Quantitative Myocardial Cytokine Expression and Activation of the Apoptotic Pathway in Patients Who Require Left Ventricular Assist Devices

Emma J. Birks, MRCP; Najma Latif, PhD; Virginia Owen, PhD; Christopher Bowles, PhD; Leanne E. Felkin, BS; Anthony J. Mullen, BS; Asghar Khaghani, FRCS; Paul J.R . Barton, PhD; Julia M. Polak, DSc; John R. Pepper, FRCS; Nicholas R. Banner, FRCP; Magdi H. Yacoub, FRS, DSc

Background—Molecular mechanisms underlying the deterioration of patients undergoing LV assist device (LVAD) implantation remain poorly understood. We studied the cytokines tumor necrosis factor (TNF)-α and interleukin (IL)-1β and IL-6 and the terminal stage of the apoptotic pathway in patients with decompensating heart failure who required LVAD support and compared them with patients with less severe heart failure undergoing elective heart transplantation.

Methods and Results—Myocardial and serum samples from 23 patients undergoing LVAD implantation were compared with those from 36 patients undergoing elective heart transplantation. Myocardial TNF-α mRNA (1.71-fold; $P<0.05$) and protein (3.43±0.19 versus 2.95±0.10 pg/mg protein; $P<0.05$) were elevated in the LVAD patients. Immunocytochemistry demonstrated TNF expression in the myocytes. Serum TNF-α was also elevated (12.5±1.9 versus 4.0±0.4 pg/mL; $P<0.0001$) in the LVAD patients. IL-6 mRNA (2.57-fold higher; $P<0.005$) and protein (27.83±9.35 versus 4.26±1.24 pg/mg protein; $P<0.001$) were higher in the LVAD candidates, as was serum IL-6 (79.3±23.6 versus 7.1±1.6 pg/mL; $P<0.0001$). Interleukin-1β mRNA expression was 9.78-fold higher in the LVAD patients ($P<0.001$). iNOS mRNA expression was similar to that in advanced heart failure patients and was not further elevated in the LVAD patients. Levels of procaspase-9 (8.02±0.91 versus 6.16±0.43 oligodeoxynucleotide [OD] units; $P<0.01$), cleaved caspase-9 (10.02±1.0 versus 7.34±0.40 OD units; $P<0.05$), intact and spliced DFF-45 (4.58±0.75 versus 2.84±0.23 OD units; $P<0.05$) were raised in LVAD patients, but caspase-3 and human nuclease CPAN were not.

Conclusions—Elevated TNF-α, IL-1β, and IL-6 and alterations in the apoptotic pathway were found in the myocardium and elevated TNF-α and IL-6 in serum of deteriorating patients who required LVAD support. These occurrences may have therapeutic implications and influence the timing of LVAD insertion. (***Circulation.*** **2001;104[suppl I]:I-233-I-240.)**

Key Words: heart-assist device ■ interleukins ■ heart failure ■ nitric oxide synthase

Left ventricular assist devices (LVADs) have become an established treatment for patients with severe heart failure. Molecular mechanisms underlying the decompensation of heart failure remain poorly understood. Understanding the mechanisms involved may help with decisions about timing of LVAD implantation and identifying new therapeutic targets in advanced heart failure.

Expression of the proinflammatory cytokine tumor necrosis factor (TNF)-α has been described in patients with chronic heart failure both in serum and myocardium,[1–5] and serum levels have been found to correlate with functional status.[2] TNF-α has been shown to produce myocardial depression both in in vitro and in vivo models.[2,3,6] Interleukin (IL)-6 also is elevated in myocardium and serum of patients with heart failure, and levels correlate with poor functional status.[1,3,7,8] IL-1β is known to cause myocardial depression in vivo[9,10] and acts synergistically with TNF-α,[10] but its role in heart failure is unclear.

TNF-α and IL-1β can activate inducible nitric oxide synthase (iNOS), and their negative inotropic effect can be mediated through iNOS induction.[11] iNOS expression has been described in patients with heart failure.[4] iNOS is a potent producer of nitric oxide, which can have a negative inotropic effect. TNF-α and IL-1β also can induce apoptosis of cardiac myocytes,[12,13] whereas IL-6 has antiapoptotic effects.[14,15]

Apoptosis is tightly regulated by the caspases, which initially are translated as inactive proenzymes and are subsequently cleaved to become active. In the end stage of the apoptotic pathway, release of cytochrome c from the mito-

From the National Heart and Lung Institute at Imperial College School of Medicine (E.J.B., V.O., C.B., L.E.F., A.J.M., A.K., P.J.R.B., J.R.P., N.R.B., M.H.Y.), Royal Brompton and Harefield Hospital, Harefield, Middlesex; and Royal Postgraduate Medical School at Imperial College School of Medicine (J.M.P.), Hammersmith Hospital, London, UK.

Correspondence to Magdi Yacoub, FRS, Professor of Cardiothoracic Surgery, Heart Science Centre, Royal Brompton and Harefield Hospital, Harefield, Middlesex UB96JH, United Kingdom. E-mail emmabirks@hotmail.com

Circulation is available at http://www.circulationaha.org